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## The dynamic niche of the foreign body reaction

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## Summary

Intracorporally implanted materials, such as medical devices, will provoke the body to initiate an inflammatory reaction. This inflammatory reaction to implanted materials is known as the foreign body reaction (FBR) and is characterized by 3 distinct phases: onset, progression, and resolution. The FBR proceeds in the creation of a dynamic microenvironment that is spatially well organized. This reaction is regulated by soluble mediators, most likely cytokines, chemokines, and matrix metalloproteinases (MMPs), which are produced locally by surrounding tissue cells and infiltrating inflammatory cells.

Key players in this process such as macrophages, PMNs, giant cells and vascular endothelial cells communicate through these secreted soluble mediators. To gain insight in the regulation of the FBR, the kinetics of soluble mediators during the FBR was studied in this thesis with focus on cytokines, chemokines, and on proteases that modify the ECM i.e. MMPs. Besides, the role of purinergic P2 receptors in the FBR was investigated. The nature of the FBR requires that the soluble mediators act in a spatial and temporally regulated manner as well. This regulation is well known for several inflammatory processes, but scarce knowledge exists about the intricate relationship between the FBR and the expression of soluble mediators. By studying these cytokines, chemokines, MMPs and P2 receptors new targets for the modulation of the FBR can be identified. Since our lab and others have found differences in FBR development between implants locations and between species, the underlying differences in cytokine, chemokine and MMP expression were determined.

Chapter 1 gives an introduction to the field of tissue engineering and describes the progress in biomaterial development. Furthermore, the stages of the FBR and the proposed role of soluble mediators in this process are discussed.

The key processes during the initiation, progression, and resolution phase are discussed in detail in Chapter 2. Since we believe that soluble mediators orchestrate the cascade of cellular processes that accompanies the FBR, the role of these mediators in cellular activation, angiogenesis, extravasation, migration, phagocytosis, and, finally, fibrosis is reviewed. Furthermore, the role of giant cells in the FBR was discussed.

It has previously been shown that the FBR in mice progresses at a much slower pace than in rats. Mice do not phagocytose subcutaneously implanted collagen, nor do they degrade collagen through extracellular routes. In contrast, rats phagocytose and degrade subcutaneously implanted collagen efficiently. Additionally, more inflammatory cells extravasate and migrate towards implanted biomaterial in rats, instead in mice extensive stroma is formed inside the biomaterial<sup>69</sup>. Although giant cells which essentially are fused macrophages, are formed during the FBR both in mice and rats, giant cells are actively involved in phagocytosis in rats only. In chapter 3 we hypothesized that the difference in FBR between the two species is caused by a difference in cytokine and chemokine expression. Therefore, cytokine and chemokine expression levels were determined in collagen disks that had been implanted subcutaneously in mice and rats in time. During the progression, genes encoding the PMN attractants CXCL1/KC and CXCL2/MIP2 were expressed higher in mice than in rats, which would explain the prolonged presence of PMNs in mice during the FBR. Additionally, the strong induction of IFN $\gamma$  in rats coincided with a higher phagocytotic activity by macrophages. Throughout the FBR the expression of TGF $\beta$  was constitutive and high in both species, but increased in mice during the progression phase. This could explain the extensive stroma formation during murine FBR. The stronger expression of TNF $\alpha$  and CCL3/MIP1 $\alpha$  in mice, unexpectedly, did not result in a high

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macrophage attraction or phagocytosis of the implanted collagen disks. Thus, most observed differences between species can be explained by the detected differences in cytokine expression. Selective inhibition of these mediators should be used to verify this relationship.

Apart from interspecies differences in FBR, the FBR also differs between implant locations. Our group previously found that collagen disks implanted on the heart were rapidly degraded, whereas as described above, the disks remain intact subcutaneously in mice. In chapter 4 we hypothesized that these differences could be a result of differences in cytokine and matrix metalloproteinase (MMP) expression. Both the higher influx of leukocytes and implant degradation correlated with a higher gene expression of pro-inflammatory cytokines such as IL-1 and IL-6, and a lower expression of the anti-inflammatory cytokine IL-10 on the heart. Furthermore, the higher supra-epicardial expression of PMN attractants CXCL1/KC and CXCL2/MIP2 correlated with a higher and prolonged PMN influx. The gene expression levels of collagen degrading MMPs, i.e. MMP8, MMP13 and MMP14 were similar in subcutaneous and supra-epicardial disks. However, the activity of these enzymes was markedly higher supra-epicardially. In addition, the MMP9 expression was higher supra-epicardially, suggesting a role for this enzyme in the degradation process. In conclusion, a strong pro-inflammatory milieu is generated after supra-epicardial implantation that enables prolonged PMN presence and activation. This, together with the high supra-epicardial MMP9 level, could explain the observed difference in Col-I degradation between locations.

As discussed in chapter 1 and 2, giant cells are a key characteristic of the FBR. Furthermore, giant cells are the predominant collagen degraders when this material is implanted subcutaneously in rats. To determine whether these giant cells are also involved in orchestration of the FBR, we analyzed the gene expression of cytokines, chemokines, angiogenic mediators and fibrotic mediator TGF $\beta$  in chapter 5. The gene expression of PMN chemoattractants, CXCL1/KC and CXCL2/MIP-2, was high in GCs micro-dissected from explanted Dacron, cross-linked collagen (HDSC), and ureido-pyrimidinone end functionalized oligocaprolactone with added RGD and PHSRN peptides (bioactive PCLdiUPy). Conversely, the gene expression levels of TGF $\beta$  and pro-angiogenic mediators VEGF and FGF were found to be low in these GCs as compared to the expression levels in total explants. GCs in bioactive PCLdiUPy displayed high cytokine and angiogenic mediator expression compared to GCs isolated from the two other studied materials, whereas chemokine gene expression in GCs isolated from HDSC was low. Thus, GCs adopt their role and expression profile in response to the material that is encountered. Controlling GC activation and inflammatory mediator production by these cells could therefore open new ways to regulate the FBR.

Apart from cytokines and chemokines, extracellular nucleotides that are released by activated and damaged cells have also been implicated as mediators of the FBR. These nucleotides can bind to the P2X and P2Y group of receptors, resulting in activation or conversely inactivation of cells. However, the local expression pattern and kinetics of these receptors at sites of inflammation are not known. Therefore, we have studied the expression of the P2 receptors expressed by inflammatory cells or by cells in the vasculature in chapter 6. In the vasculature, the expression of P2X7R, P2Y1R, and P2Y2R increase until day 2. The expression of P2X7R and P2Y1R on macrophages and giant cells increased during the course of the inflammatory reaction which was studied for 21 days. The expression of the P2Y2R on macrophages and giant cells inside the foreign body increases with time, whereas the expression on

macrophages in the surrounding tissue is maximal at day 5. The expression of P2X1R remains at a constant low level. The upregulation of P2X7R, P2Y1R, and P2Y2R over time suggests a regulatory function for these receptors in inflammation. Inhibition of these receptors during the FBR could prove their role in this response.

In chapter 8 the results presented in this thesis are discussed. This thesis shows that the outcome of the dynamic niche of the FBR strongly differs between implant locations and between species. Depending on the location of implantation PMNs, macrophages and giant cells are central determinants of the FBR. Giant cells showed to express high levels of cytokines and chemokines, and this expression pattern was adapted in response to the biomaterial encountered. We shows that despite a stronger pro-inflammatory niche in mice than in rats, giant cells (or macrophages for that matter) in mice do not degrade subcutaneously implanted collagen as is observed in rats. To elucidate this future research has to focus on the differences in MMP expression (especially MMP8 and MMP9) observed. Knowledge of the local inflammatory niche induced by a biomaterial positioned in the inflammatory response due to tissue damage opens the way to develop smart (e.g. factor based) materials which will functionally regenerate tissues and offer many possibilities for future regenerative medicine.